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Award Number: DAMD17-92-C-2066

TITLE: Evaluation of Biomonitoring Systems for Assessment of Contaminated Waters and Sediments at U.S. Army Installations

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REPORT DATE: December 1999

TYPE OF REPORT: Final

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. AGENCY USE ONLY *(Leave Blank)*

2. REPORT DATE
December 1999

3. REPORT TYPE AND DATES COVERED
FINAL (18 Jun 92 - 31 Dec 99)

4. TITLE AND SUBTITLE
Evaluation of Biomonitoring Systems for Assessment of Contaminated Waters and Sediments at U.S. Army Installations

5. FUNDING NUMBERS
DAMD17-92-C-2066

6. AUTHORS
Dennis T. Burton, Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
University of Maryland
Wye Research and Education Center
Queenstown, Maryland 21658

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S Army Medical Research and Materiel Command
Fort Detrick, MD 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for public release; distribution unlimited.

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200 words)*

The primary objective of the study was to evaluate the use of several biomonitoring systems for hazard/risk assessment of potentially contaminated waters and sediment-associated contaminants at U.S. Army Installations. An array of toxicity tests structured in a tiered framework, which ranged from a rapid acute test of 5 minutes in duration to a 9-month chronic carcinogenicity assays was evaluated. Both aqueous and sediment phase tests were evaluated. The studies were structured, where possible, to provide toxicity system evaluation data as well as data for various Superfund sites at the Aberdeen Proving Ground, Aberdeen, Maryland. Most screening-level Superfund ecological risk assessments require the use of ecotoxicity values which provide chronic no-observed-adverse-effect-level (NOAEL) data for exposures to contaminants. Several of the biomonitoring systems evaluated in the current study provided appropriate NOAEL data. Other assays such as, the Ames mutagenicity assay, frog teratogenesis assay-Xenopus (FETAX); and Japanese medaka (Oryzias latipes) carcinogenicity assay are not normally structured to provide NOAEL endpoints. The use of the systems, however, as weight-of-evidence for ecological hazard/risk assessment is presented for various Superfund sites. Likewise, the results of the studies used for regulatory and compliance activities by the Army, EPA, and State of Maryland regulators are presented in the report.

14. SUBJECT TERMS
Microtox®, Rotoxkit®, Ceriodaphnia dubia, Pimephales promelas, Lepomis macrochirus, Oryzias latipes, Selenastrum capricornutum, Ames assay, SCE assay, FETAX; O. latipes carcinogen assay, Leptocheirus plumulosus, Hyalella azteca, ecoloical risk assessment, Superfund, biomonitoring, bluegill ventilatory system

15. NUMBER OF PAGES
40

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

NSN 7540-01-280-5500

Standard Form 298 (Rev. 2-89)
Prescribed by ANSI Std. Z39-1
298-102

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D.T. Burton 1-31-00

PI - Signature Date

Certification of Technical Data Conformity (May 1987)

The contractor, University of Maryland at College Park, hereby certifies that, to the best of its knowledge and belief, the technical data delivered herewith under Contract DAMD17-92-C-2066 is complete, accurate, and complies with all requirements of the contractor.

Date: January 31, 2000

Name and Title of Certifying Official: D. T. Burton

Dennis T. Burton, Ph.D.
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EXECUTIVE SUMMARY

A primary objective of the study was to evaluate the use of several biomonitoring systems for hazard/risk assessment of potentially contaminated waters and sediment-associated contaminants at U.S. Army installations. An array of toxicity tests structured in a tiered framework, which ranged from a rapid acute test of 5 min in duration (Microtox®) to a 9-month chronic carcinogenicity test, was evaluated. Several levels of biological organization were studied. They included detection endpoints as diverse as changes in microbial bioluminescent activity to whole animal growth and reproduction. Several of the test systems evaluated, such as, mutagenicity, teratogenicity, and carcinogenicity of contaminated media, were not standard test systems normally recommended by the U.S. Environmental Protection Agency (EPA) for ecological risk assessment at Superfund sites. A secondary objective of the study was to perform as many of the biomonitoring tests as possible in real-time in the field. In addition to the toxicity evaluation and monitoring data obtained, an evaluation of the field deployment of the U.S. Army Center for Environmental Health Research's (USACEHR) acute toxicity biomonitoring system was made.

The following array of aqueous phase test systems, some of which were standard EPA tests, were used in the evaluation: (1) Bacterial respiration– 5- or 15-min EC50 Microtox® (*Photobacterium phosphorum*) assay; (2) Acute lethality– 24-h LC50 Rotoxkit® (*Brachionus rubens*) assay, 48-h LC50 cladoceran (*Ceriodaphnia dubia*) bioassay, 96-h fathead minnow (*Pimephales promelas*) bioassay, 96-h LC50 bluegill (*Lepomis macrochirus*) bioassay, and 96-h Japanese medaka (*Oryzias latipes*) assay; (3) Algal growth– 96-h green alga (*Selenastrum capricornutum*) bioassay; (4) Short-term chronic survival, reproduction, and growth– 7-d cladoceran (*C. dubia*) survival and reproduction test and 7-d fathead minnow (*P. promelas*) survival and growth test; (5) Mutagenicity– Ames *Salmonella*/mammalian-microsome reverse assay for gene mutation and the sister chromatid exchange (SCE) assay for primary DNA damage; (6) Teratogenicity– 96-h frog teratogenesis assay-*Xenopus* (FETAX); and (7) Carcinogenicity– 6- and 9-month Japanese medaka (*O. latipes*) carcinogen model. The following sediment phase tests were evaluated: (1) acute lethality– 10-d infaunal amphipod (*Leptocheirus plumulosus*) bioassay and 10-d epifaunal amphipod (*Hyalella azteca*) bioassay and (2) chronic survival, growth, and reproduction– 28-d *L. plumulosus* bioassay and 28-d *Hyalella azteca* bioassay.

Most of the above aqueous phase test systems provided information that was useful in the toxicological evaluation of groundwater contaminated with heavy metals and chlorinated volatile organic compounds (VOCs). The 5-min EC50 Microtox® assay proved to be a sensitive and easily run (1-h test) assay for toxicity. It was particularly useful for frequent monitoring of long-term groundwater toxicity tests which ran from 6 to 9 months in duration. Among the suite of acute invertebrate and fish bioassays, it was found that all systems provided data which encompassed a range of sensitivities to the contaminant mixture. The 24-h LC50 Rotoxkit® assay with the rotifer (*B. rubens*), which was the fastest

test in the suite to conduct, was the least sensitive test for groundwater containing heavy metals and VOCs.

No mutagenicity (Ames assay) occurred in unconcentrated groundwater samples containing heavy metals and VOCs or in groundwater samples concentrated 10X. A positive response was found for groundwater in the sister chromatid exchange assay when the groundwater samples were concentrated ≈50,000X. The use of a ≈50,000X response in an ecological risk assessment is questionable. The FETAX teratogenicity assay proved to be important in detecting malformations in contaminated groundwater (heavy metals and VOCs) and contaminated surface water (heavy metals from soil runoff) from an area in which previous mortality studies (teratogenicity was not evaluated) found no effect to frogs. A 6- and 9-month carcinogenicity assay was run with groundwater contaminated with heavy metals and VOCs. Hepatocellular neoplasms were observed in Japanese medaka exposed in both a 6- and 9-month protocol. In general the number of hepatocellular neoplasms observed at nine months was greater than the number observed at six months in both male and female medaka. The increased numbers of hepatocellular neoplasms at nine months were qualitatively higher; but, was not statistically significant from the spontaneous neoplasms in the controls. The number of hepatocellular neoplasms in fish initiated with 10 mg/L diethylnitrosamine was greater at nine months than the number observed at six months. Thus, the longer exposure time is important when evaluating groundwater that has a low potential to cause hepatic neoplasms.

Chronic 28-d sediment toxicity studies with *L. plumulosus* and *H. azteca* exposed to wetland sediment contaminated with heavy metals showed that the sediments were not toxic. Bulk chemical analyses of the sediment revealed high concentrations of several heavy metals were present. National Oceanic and Atmospheric Administration sediment threshold values for heavy metals indicated that the sediment may be toxic to benthic organisms. The lack of toxicity appears to be a function of the simultaneously extractable metal/acid volatile sulfide (SEM/AVS) ratios in the exposure sediments; not the bulk chemicals present. In all cases, the SEM/AVS ratios were <1 which show that the divalent heavy metals in the groundwater are bound and thus are not bioavailable.

The evaluations of the above biomonitoring systems were conducted at various Superfund sites at the Edgewood Area of the Aberdeen Proving Ground (APG), Aberdeen, Maryland. The studies were structured, where possible, to provide toxicity system evaluation data for USACEHR as well as data for the APG Installation Restoration Program for environmental evaluations of various Superfund sites. Most screening-level EPA Superfund ecological risk assessments require the use of ecotoxicity values which provide chronic no-observed-adverse-effect-level (NOAEL) data for exposures to contaminants. Ecological effects of most concern are those that cause adverse effects on survivorship, development, and reproduction of populations. Several of the biomonitoring systems evaluated in the current study provide appropriate NOAEL data. The test protocols for the mutagenicity and carcinogenicity assays are not structured to provide NOAEL endpoints.

However, the assays provide important weight-of-evidence data that are not routinely required for Superfund screening-level ecological risk assessments.

Several ecological hazard/risk assessments at various Superfund sites using the above biomonitoring systems have been reported in various Annual Reports and/or Interim Reports which have been entered in the Department of Defense Technical Reports Database. Three additional studies are currently being finalized and will be submitted to the Database as Supplemental Reports. The use of each study by the Army for regulatory and compliance activities is briefly described below:

Biomonitoring and Hazard Assessment Evaluation of Contaminated Groundwater at Beach Point, Edgewood Area, Aberdeen Proving Ground

The biomonitoring and hazard assessment evaluation of contaminated groundwater at Beach Point study was used as part of the APG Installation Restoration Program Beach Point Superfund remedial investigation/feasibility study (RI/FS). The RI/FS concluded that no environmental risks exist at Beach Point. An institutional control action at Beach Point was granted in the Record of Decision. The RI/FS cost-benefit analyses estimated that the potential cost savings for institutional control versus the most expensive alternative pump and treat scenarios to meet drinking water standards would be (in 1996 dollars) $4,000,000 to $5,000,000 in initial costs with annual operation and maintenance costs of ~$500,000.

Evaluation of Contaminated Groundwater at West Branch of Canal Creek, Edgewood Area, Aberdeen Proving Ground

The toxicological evaluation of the contaminated groundwater at West Branch of Canal Creek showed that the *in situ* groundwater was toxic to several trophic levels. However, an evaluation as the groundwater as it moves through the wetland sediments showed that the media was not toxic to benthic organisms. The simultaneously extractable metal/acid volatile sulfide (SEM/AVS) ratios in the exposure sediments were <1 in all cases which indicated that the divalent heavy metals in the groundwater were not likely toxic. Based on the findings of this study and a VOC study by USGS, it was recommended to the Army that they consider the wetland as a natural remediation process. The recommendation is currently being considered as a remediation option for the aquifer.

Continuous Acute Toxicity Biomonitoring of Old O-Field Groundwater Treatment Facility Effluent, Edgewood Area, Aberdeen Proving Ground

The continuous acute toxicity biomonitoring of the Old O-Field Groundwater Treatment Facility Effluent (GWTF) demonstrated that the operation of the biomonitoring system has had a positive effect on the management of the GWTF operation. After an initial learning period when the biomonitoring system was adapted to the specific operational conditions at the GWTF, the engineers at the facility became more aware of

effluent conditions. As a result, the biomonitoring stress alarms have not unduly disrupted effluent treatment operations. Historically, the system facilitated signing of the Record of Decision for the site because it showed that the groundwater could be safely treated before discharge. A cost-benefits analysis estimated that the system initially saved $300,000 to $400,000 by eliminating treatment studies requested by EPA and State of Maryland regulators.

Toxicity Evaluation of Selected Sites During High Surficial Aquifer Flow at J-Field, Edgewood Area, Aberdeen Proving Ground

The toxicity evaluation of selected sites during high surficial aquifer flow at J-Field supported the toxicological findings of previous studies. Additional information was obtained using the FETAX assay. Prior screening studies at J-Field concluded that no adverse ecological effects would occur to indigenous frogs. The current study showed, however, that malformations occurred to frogs at all marsh study sites. The most heavily contaminated well upgradient from the J-Field Pushout Area contained toxic concentrations of chlorinated VOCs; however, chemical data from the current study for the marsh sites indicated that heavy metals were the primary contaminants of concern in the boundary area of the marsh. The current study supported Argonne National Laboratory's draft Remedial Investigation conclusion that ecological receptors are at risk in the marsh adjacent to the TBP Pushout Area. To date, the Army has performed soil removal actions in the TBP Pushout Area and is continuing an investigating of the use of phyto-remediation techniques to reduce the high VOC concentrations in the groundwater.

Toxicity of Diisopropyl Methylphosphonate (DIMP) to Aquatic Organisms at the Building E3640 Process Laboratory Site, Edgewood Area, Aberdeen Proving Ground

Diisopropyl methylphosphonate (DIMP) is present in the subsurface soils and surficial groundwater in the vicinity of the Building E3640 Process Laboratory. The plume is migrating towards the Kings Creek ecosystem. The concentrations of DIMP in the groundwater at the Building E3640Process Laboratory were found to be an order of magnitude or lower than the no-observed-adverse-effect levels (NOAEL) for short-term chronic exposure to the parent compound. The NOAELs for the alga (reduction in growth), invertebrate (reduction in neonate production), frog embryo (mortality), and larval fish (reduction in growth) were 711, 142, 398, and 142 mg/L, respectively. Based of the NOAELs, it was concluded that the plume of DIMP migrating in the surficial aquifer from the Building E3640 Process Laboratory area towards Kings Creek should have minimal impact on the aquatic environment. The toxicity data obtained in the study were used as part of a data base for a screening-level ecological risk assessment which is presented below.

Screening-Level Ecological Risk Assessment of Diisopropyl Methylphosphonate (DIMP) at the Building E3640 Process Laboratory Site, Edgewood Area, Aberdeen Proving Ground

The screening-level ecological risk of diisopropyl methylphosphonate (DIMP) at the Building E3640 Process Laboratory Site showed that DIMP poses a negligible risk to plants and animals in the Process Laboratory and Kings Creek areas. The conclusion was based on the fact that the hazard quotients (HQ) of all potential receptors in the study area were <1. The HQs for soil microorganisms, soil and litter invertebrates, and terrestrial plants were estimated to be 0.06, 0.05, and 0.60, respectively. The HQs for aquatic microorganisms (bacteria), aquatic algae, aquatic invertebrates, fish, and amphibians were 0.06, 0.01, 0.10, 0.04, and 0.02, respectively. The HQs for birds and mammals were 0.63 and 0.76, respectively. No DIMP data were available for reptiles; thus, an HQ was not calculated for reptiles. Based on the data for other vertebrates, the weight-of-evidence suggests that DIMP will not be a risk to reptiles. At the writing of this report, the EPA Region 3 and the State of Maryland Department of the Environment have agreed with the Army that DIMP poses no ecological hazard to the environment.

Bioaccumulation of Total Mercury and Methylmercury in Earthworms and the Ecological Risk to Birds and Mammals at the Northeast Test Hut, Graces Quarters, Aberdeen Proving Ground

The bioaccumulation of total mercury and monomethylmercury (MMHg) was determined in earthworms exposed to four soils with various concentrations of the chemicals. Soils with the highest concentration of total mercury and MMHg at Graces Quarter's Northeast Test Hut was labeled high, followed by soils with intermediate, low, and reference concentrations. The bioaccumulation factors (BAFs) for total mercury in the high, intermediate, low, and reference treatments were 0.8, 0.6, 6.0, and 3.7, respectively. The BAFs for MMHg were 175, 188, 234, 262, respectively, in the high, intermediate, low, and reference treatments. The risk to the American robin and short-tailed shrew, which feed on earthworms exposed to MMHg, was evaluated using (1) a conservative diet of 100% earthworm containing MMHg and (2) a more realistic diet of 22 and 32% earthworms for robins and shrews in the mid-Atlantic region. Environmental effects quotients (EEQs) were calculated under both of the above scenarios at the four soil concentrations. The EEQs for the robin at a 100% earthworm diet indicated that the potential for adverse effects exists if the robin consumes earthworms which bioaccumulate MMHg at the high and intermediate soil sites at the Northeast Test Hut. The EEQs at the low and reference sites indicate that no adverse effects will occur when the diet is 100% earthworms. The EEQs based on the 22% earthworm diet at all soil concentrations indicate that no adverse effect is likely to occur to the robin. The EEQs for the shrew at both the 100% and 32% earthworm diet indicate that the potential adverse effects from MMHg in the soils is very low. The results of the study are currently being reviewed by EPA and State of Maryland regulators.

ACKNOWLEDGMENTS

I thank Mr. Tommy Shedd (COR) of the U.S. Army Center for Environmental Health Research, Fort Detrick, Frederick, Maryland, for his continual support and help throughout the study. I would also like to acknowledge all those individuals listed in the Annual/ Interim Reports given in the Reference Section who provided help with various phases of the studies. I acknowledge USACEHR for supporting the study through U.S. Army Contract No. DAMD17-92-C-2066.

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LIST OF PUBLICATIONS AND MEETING ABSTRACTS

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LIST OF STUDY PERSONNEL

Listed below, as required for all Final Reports submitted to The U.S. Army Medical Research and Materiel Command, are the individuals who were supported, in whole or part, from funds awarded by USACEHR for the research project:

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1. INTRODUCTION

A primary objective of the study was to evaluate the use of several biomonitoring systems for hazard/risk assessment of potentially contaminated waters and sediment-associated contaminants at U.S. Army installations. An array of toxicity tests structured in a tiered framework, which ranged from a rapid acute test of 5 min in duration (Microtox®) to a 9-month chronic carcinogenicity test, was evaluated. Several levels of biological organization were studied. They included detection endpoints as diverse as changes in microbial bioluminescent activity to whole animal growth and reproduction. Several of the test systems evaluated, such as, mutagenicity, teratogenicity, and carcinogenicity of contaminated media, were not standard test systems normally recommended by the U.S. Environmental Protection Agency (EPA) for ecological risk assessment at Superfund sites. A secondary objective of the evaluation phase of the study was to perform as many of the biomonitoring tests as possible in real-time in the field. In addition to the toxicity evaluation and monitoring data obtained, an evaluation of the field deployment of the U.S. Army Center for Environmental Health Research's (USACEHR) acute toxicity biomonitoring system was also made.

All of the biomonitoring system evaluations were conducted at various Superfund sites at the Edgewood Area of the Aberdeen Proving Ground (APG), Aberdeen, Maryland. The studies were structured, where possible, to provide toxicity system evaluation data for USACEHR as well as data for the APG Installation Restoration Program for environmental evaluations of various Superfund sites. Most of the ecological hazard/risk assessments at the Superfund sites where the biomonitoring systems were studied have been reported in Annual Reports and/or Interim Reports which have been entered in the Department of Defense Technical Reports Database. In addition to the studies which have been published in the Department of Defense Technical Reports Database, three studies are currently being reviewed by EPA and the State of Maryland and will be published as Supplemental Reports in the database. A summary of all published and unpublished studies is presented in this report. For ease of presentation, each study unit is given in a separate section.

2. BIOMONITORING AND HAZARD ASSESSMENT EVALUATION OF CONTAMINATED GROUNDWATER AT BEACH POINT

The primary objective of the study was to evaluate the use of several biomonitoring systems for assessing contaminated groundwater. Contaminated groundwater, which contained multiple heavy metals and chlorinated aliphatic hydrocarbons, was studied. The contaminated groundwater was withdrawn from the lower depths of the surficial aquifer at Beach Point which is located in the Canal Creek Area of the U.S. Army Aberdeen Proving Ground-Edgewood Area, Aberdeen, MD. Groundwater was withdrawn from well CC-33B, which is the most the highly contaminated well at Beach Point. A hazard assessment of the groundwater release into the Bush River was performed using the biomonitoring results. The results of the study are given in Burton et al. (1994a,b).

2.1 Biomonitoring Evaluation

Several EPA priority pollutant heavy metals were found in the groundwater at Beach Point. The concentrations of cadmium, copper, nickel, and zinc exceeded one or more of EPA's numerical water quality criteria for the specific metal. A number of chlorinated aliphatic compounds were also found in the groundwater, several of which are EPA priority pollutants. The chlorinated aliphatic organics that occur at the lower depths of the aquifer may be present as a residual denser-than-water nonaqueous phase liquid (DNAPL). Of the organics present in the groundwater, none had octanol water partition coefficients (log K_{ow} or log P) >3; thus, bioaccumulation was not a potential toxicological problem.

An array of nine biomonitoring systems integrated into a tiered hazard framework was evaluated during a 9-month study. The biomonitoring systems included a number of endpoints. The pH of the groundwater from well CC-33B was 4 (± 0.1); thus, many of the assays were conducted at both pH 4 and pH 7. The toxicity at pH 7 was studied so that the data could be used in the hazard assessment of the groundwater as it entered the Bush River which has pH values close to the neutral range.

Toxicity was detected at various groundwater concentrations by 7 of 9 biomonitoring systems. The Ames assay for mutagenicity was negative in all cases (1, 10, and 100% groundwater by volume). Japanese medaka (*Oryzias latipes*) growth was not affected by 9 months of exposure to 1 and 10% groundwater by volume in a chronic histopathology assay. No significant lesions were found in the Japanese medaka exposed to groundwater concentrations up to 10% groundwater by volume (highest concentration studied). A positive response was found for 100% groundwater in a sister chromosome exchange (SCE) assay for DNA damage when the groundwater sample was concentrated ≈50,000X. The SCE assay was negative for unconcentrated 100% groundwater. The positive SCE response in the 50,000X concentrated sample was judged not to be important to aquatic organisms in the receiving stream.

The lowest concentration of groundwater that caused no-observed-adverse-effect at pH 4, in the test systems in which the no-observed-adverse-effect-level NOAEL value could be determined, was 10% groundwater by volume. A NOAEL of 10% groundwater by volume occurred in 3 out of 4 tests in both a 7-d cladoceran (*Ceriodaphnia dubia*) and a 96-h frog (*Xenopus laevis*) embryo teratogenesis assay - *Xenopus* (FETAX); it occurred once in a 7-d fathead minnow (*Pimephales promelas*) test. The NOAEL concentration was higher at pH 7 in both the fathead minnow and FETAX assays. The 10% groundwater by volume NOAEL for the cladoceran at pH 4, however, did not change when the organism was exposed to buffered groundwater at pH 7.

When estimated maximum acceptable toxicant concentrations (MATC) were established, the data for alga (*Selenastrum capricornutum*), invertebrate (*C. dubia*), and fish (*P. promelas*) used in the biomonitoring study suggested that the groundwater would not be harmful at a concentration of 10% groundwater by volume. Likewise, no genotoxicity, developmental toxicity, or chronic histopathology occurred at 10% groundwater by volume. Thus, the biomonitoring data suggested that chronic toxicity may not occur in the Bush River at a dilution of 10:1. Chronic toxicity was predicted to occur in the Bush River if the groundwater entered the receiving stream at the concentrations which occur in well CC-33B.

2.2 Hazard Assessment Evaluation

A number of worst-case assumptions were made for the Beach Point hazard assessment when sufficient data were not available. The flowing assumptions were made for the calculation of the estimated discharge rate of the surficial aquifer into the Bush River. The surficial aquifer was considered to be homogeneous, characterized by isotropic flow conditions. The average saturated thickness of the aquifer was assumed to be 18.8 m (61.8 ft). The aquifer was assumed to discharge the entire length of the Beach Point Peninsula fronting the Bush River; a more realistic estimate of length of discharge from Beach Point is probably one third to one half, rather than the entire length.

With regard to the contaminant concentrations in the groundwater, the assumption was made for heavy metals that no retardation occurred via adsorption onto solid surfaces or trapping by clays through ion exchange. It was also assumed that no precipitation of the metals occurred when the pH shifted from 4 to neutrality when the groundwater entered the receiving stream. The assumption was made for the DNAPLs that no abiotic (chemical) or biotic (microbial) transformations occurred.

It was also assumed that the heavy metals and residual DNAPLs were homogeneously mixed throughout the complete aquifer at the highest concentration (not the average concentration) measured during the course of the study. It was assumed that the maximum concentrations of materials would not increase above those currently present in well CC-33B since the original sources of the contaminants were not longer present. The assumption was made that the highest concentrations of the heavy metals and

chlorinated aliphatics in the aquifer all moved through the sediments into the Bush River without any biotransformations or other physical/chemical transformations occurring in the sediment or water column of the Bush River.

When the above assumptions were used in the hazard assessment, the groundwater may be considered to be a potentially hazardous material to the benthic biota of the Bush River. The hazard to water column aquatic biota would rapidly dissipate as the groundwater materials mix in the receiving stream. Because the potential water quality impacts were judged to be minimal, a mixing zone approach by the State of Maryland which allows for local exceedences of water quality standards may be pursued. The State of Maryland Code (COMAR) allows for a mixing zone on a case-by-case basis. Pollutant concentrations within a mixing zone may exceed the specified water quality standards within a localized vicinity of an outfall.

Near-field (ULINE model) and far-field (dye-tracer model with input data originally obtained for the Bush River) EPA-approved screening level dilution models were run to estimate the dilution of the groundwater discharge plume in the Bush River. The concentrations of the groundwater hazardous substances were estimated for the near-field and far-field in the Bush River using the mixing zone requirements of the State of Maryland.

The model analyses showed that a total near-field dilution (near-field dilution corrected for the influence of far-field dilution) of approximately 42:1 for the application of the State's acute aquatic life criteria and a near-field dilution level of 168:1 for the application of chronic criteria would occur. Thus, contaminants introduced via Beach Point groundwater into Bush River receiving waters at a concentration of 42 µg/L would be diluted locally to a concentration of approximately 1 µg/L or less. When the dilution factors were applied to groundwater quality at Beach Point, none of the heavy metals or chlorinated aliphatic compounds exceeded Maryland's current acute or chronic aquatic life criteria. The dilution study showed that detectable concentrations of some of the chlorinated organics would occur in the receiving stream when the conservative assumptions concerning the groundwater contaminants were used in the model.

The results of the study were used as part of the APG Installation Restoration Program Beach Point Superfund remedial investigation/feasibility study (RI/FS). The RI/FS concluded that no environmental risks exist at Beach Point. An institutional control action at Beach Point was granted in the Record of Decision. The RI/FS cost-benefit analyses estimated that the potential cost savings for institutional control versus the most expensive alternative pump and treat scenarios to meet drinking water standards would be (in 1996 dollars) $4,000,000 to $5,000,000 in initial costs with annual operation and maintenance costs of ~$500,000.

3. EVALUATION OF CONTAMINATED GROUNDWATER AT WEST BRANCH OF CANAL CREEK

The toxicological evaluation of groundwater contamination at the U.S. Army Aberdeen Proving Ground-Edgewood Area, West Branch of Canal Creek Area (WBCC), Aberdeen, MD, was designed to be conducted in two sequential phases. Phase 1 was a determination of the potential toxicity of the groundwater *in situ*. If the Phase 1 evaluation showed that the groundwater was not toxic, further hazard assessment studies of the WBCC ecosystem would not be necessary. Phase 2, an evaluation of the potential toxicity of the groundwater as it moves through the WBCC wetland sediments, was to be implemented if the groundwater proved to be toxic. The results of the studies are given in Burton et al. (1995).

3.1 Phase 1 Groundwater Toxicity

The primary objective of Phase 1 was to evaluate the potential toxicity of the groundwater *in situ* to aquatic organisms because it was known that the groundwater entered the West Branch of Canal Creek ecosystem. Although microorganisms are the primary organisms present in most subsurface environments, an array of surrogate biomonitoring systems integrated into a tiered hazard framework was used in the evaluation. An array of biomonitoring assays covering several levels of biological complexity was used to maximize predictability of potential adverse pollutant effects to aquatic organisms during a 9-month evaluation. A secondary objective of Phase 1 was to evaluate, where test systems were appropriate for use in low salinity waters, the potential toxicity of West Branch of Canal Creek water. The West Branch of Canal Creek studies were conducted concurrently with the groundwater studies to obtain background data on the potential toxicity of the creek water. Only aqueous phase assays were used in the water column studies of West Branch of Canal Creek water; no sediment systems were studied.

The contaminated groundwater in the West Branch of Canal Creek study area contains multiple heavy metals and chlorinated aliphatic hydrocarbons. Groundwater was withdrawn from well CC-27B, which is one of the two most highly contaminated wells located in the Canal Creek aquifer at the West Branch of Canal Creek site.

Several EPA priority pollutant heavy metals were found in the groundwater. Copper, mercury, and silver concentrations in the groundwater exceeded, in one or more tests, the U.S. Environmental Protection Agency's (EPA) numerical water quality criteria for the specific metal. Aluminum was also present at high concentrations in the groundwater; however, EPA has not finalized their draft numerical water quality criteria for the metal. Thus, it is not clear whether or not the concentrations in the groundwater may exceed EPA's numerical water quality criteria for aluminum.

Thirteen chlorinated aliphatic compounds were found in the groundwater, several of which are EPA priority pollutants. None of the priority pollutant organics found in the groundwater currently have numerical water quality criteria values; however, lowest observed effect levels (LOEL) for several of the compounds are available. All of the LOELs are one or more orders of magnitude higher than the concentrations found in the groundwater.

Eleven of the 13 volatile organics found in the groundwater had octanol water partition coefficients (log K_{ow} or log P) less than 3. Thus, bioaccumulation was not considered to be a potential toxicological problem for most of the volatile organics present in the groundwater. 1,2-Dichlorobenzene and 1,2,4-trichloro-benzene, which have K_{ows} of 3.4 and 4.2, respectively, were found in only one groundwater sample at the beginning of the study. Thus, it is difficult to determine how important bioaccumulation may be for the two compounds.

An array of eight biomonitoring systems integrated into a tiered hazard framework was used in the 9-month study. The biomonitoring systems included a number of endpoints. The pH of the groundwater from well CC-27B was ≈4; thus, many of the assays were conducted at both pH 4 and pH 7. The toxicity at pH 7 was studied so that the data could be used, if necessary, in the Phase 2 hazard assessment of the groundwater as it enters the West Branch of Canal Creek which has pH values close to the neutral range.

Toxicity was detected at various groundwater concentrations by 6 of the 8 biomonitoring systems. The Ames assay for mutagenicity was negative in all cases for groundwater, West Branch of Canal Creek water, and filtered APG-EA tap water. Differences in Japanese medaka (*Oryzias latipes*) growth were found in a chronic 9-month histopathology assay when the fish were exposed to 1, 5 and 25% groundwater by volume diluted with either APG-EA dechlorinated tap water or West Branch of Canal Creek surface water. In general, the fish were smaller when grown in groundwater diluted with West Branch of Canal Creek water compared to those reared in groundwater diluted with APG-EA dechlorinated tap water. Most females were larger than males when reared in groundwater diluted with either West Branch of Canal Creek water or APG-EA dechlorinated tap water.

Experimental Pathology Laboratories, Inc. (EPL), Herndon, VA, analyzed the Japanese medaka in the chronic nine-month study for incidences of hepatocellular neoplasia, neoplasms other than hepatocellular neoplasms, and non-neoplastic lesions and concluded the following. "...at nine months among male and female medaka there was no effect of groundwater on the incidence of hepatocellular neoplasia [at concentrations up to 25% groundwater by volume (highest concentrations studied) when APG-EA dechlorinated tap water was used as diluent water]." "At nine months among the males there was a slight effect of 25% groundwater concentration on the incidence of hepatocellular neoplasia...[and]...among the females there was no effect of groundwater

exposure on hepatocellular neoplasia [when West Branch of Canal Creek water was used as diluent water for six months and dechlorinated tap water for three additional months]."

EPL found the following at the end of the nine-month study when Japanese medaka were initiated for 48 h at 13 days of age with 10 mg/L diethylnitrosamine (DEN). "At nine months there appeared to be a promotional effect of the groundwater at 25% concentration on hepatocellular neoplasia in male medaka (12 of 29 fish affected), although eight of 40 control medaka also had hepatocellular neoplasia at nine months [in fish exposed to 25% groundwater by volume diluted with APG-EA dechlorinated tap water]." "At nine months there appeared to be a trend of increasing percentage of hepatocellular neoplasms from controls in 25% groundwater, but the differences between groups in number of neoplasms was not great."

In DEN-initiated fish exposed to West Branch of Canal Creek water for six months followed by three months of exposure to groundwater in APG-EA dechlorinated tap water, EPL concluded "At nine months among male medaka there appeared to be a promotional effect of the groundwater on hepatocellular neoplasia based on the apparently low incidence of hepatocellular neoplasms in controls...This low incidence may be spurious..." "At six months among female medaka there appeared to be a promotional effect of the Canal Creek water on hepatocellular neoplasia. At six and nine months among female medaka there was no effect of the groundwater on hepatocellular neoplasia. The number of medaka with hepatocellular neoplasia increased at nine months over six months in all groups and at nine months the incidence was greatest among control Groups..."

The groundwater was acutely toxic at pH 4 to a green alga (*Selenastrum capricornutum*), cladoceran (*Ceriodaphnia dubia*), fathead minnow (*Pimephales promelas*), and Japanese medaka. From an acute toxicity standpoint, the groundwater appeared to be less toxic to the green alga at pH 7. The groundwater was not acutely toxic at pH 7 to the cladoceran, fathead minnow, or Japanese medaka.

The lowest concentration of groundwater that caused no observable adverse effect (NOAEL; no-observed-adverse-effect-level) at pH 4, in the test systems in which the NOAEL value could be determined, was 10% groundwater by volume. A NOAEL of 10% groundwater by volume occurred in 3 out of 5 tests for the green alga (*S. capricornutum*); 4 out of 5 tests in both a 7-d cladoceran (*C. dubia*) and a 96-h frog (*Xenopus laevis*) embryo teratogenesis assay - *Xenopus* (FETAX). A NOAEL of 18% groundwater by volume occurred in 2 of 5 tests in a 7-d fathead minnow (*P. promelas*) test. The groundwater was not toxic at pH 7 in the 7-d fathead minnow test and in 2 of 5 FETAX assays. The NOAEL (18% groundwater by volume) was higher at pH 7 in 3 of the 5 FETAX assays. The 10% groundwater by volume NOAEL for the green alga and cladoceran at pH 4, however, was essentially the same when the organisms were exposed to buffered groundwater at pH 7.

3.2 Phase 2 Wetland Sediment Toxicity

Phase 2 of the evaluation was initiated to determine the potential hazard of the groundwater to aquatic organisms as it moved through the wetland sediments into WBCC because toxicity was found in Phase 1. Biodegradation studies of the groundwater VOCs at the site by the U.S. Geological Survey (USGS) showed that the majority of each compound was biodegraded as it was transported up through the wetland sediments toward the sediment surface of the creek. In most cases, the parent compounds were not detected in the surface water. As a result of the USGS VOC findings and the fact that the heavy metals in the groundwater could be bound (metals not bioavailable) in the sediment via acid volatile sulfides, the Phase 2 toxicological evaluation was directed toward sediment organisms which would be the most likely impacted aquatic group. The assumption was made that if the benthic species were not impacted, pelagic species would not be impacted by the groundwater.

Chronic sediment toxicity studies (28-d survival, growth and reproduction assays) of an infaunal (*Leptocheirus plumulosus*) and epifaunal (*Hyalella azteca*) amphipod exposed to WBCC sediment showed that the sediments were not toxic. The simultaneously extractable metal/acid volatile sulfide (SEM/AVS) ratios in the exposure sediments were <1 in all cases which indicated that the divalent heavy metals in the groundwater were not bioavailable. The lack of toxicity to the benthic test organisms showed that the binding of metals and biodegradation of the VOCs were significant natural attenuation mechanisms in the wetland sediments. Phase 2 of the study indicated that the wetland area of WBCC serves as an unique natural remediation process. Based on the findings of this study and the VOC study by the USGS, it was recommended to the Army that they consider the wetland as a natural remediation process. The recommendation is currently being considered as a remediation option for the aquifer at WBCC.

4. CONTINUOUS ACUTE TOXICITY BIOMONITORING OF OLD O-FIELD GROUNDWATER TREATMENT FACILITY EFFLUENT

Old O-Field is a hazardous waste and ordinance disposal site which contaminated the underlying confined groundwater aquifer. Wells have been placed downgradient in the aquifer to capture the contaminated groundwater before it enters Parish Creek which flows into the Gunpowder River. The contaminated groundwater is collected and treated at the Old O-Field Groundwater Treatment Facility (GWTF) with subsequent discharge to the Gunpowder River. An in-line automated fish ventilatory biomonitoring system developed by the U.S. Army Center for Environmental Health Research (USACEHR) was installed at the GWTF in the first quarter of CY95 to monitor the effluent for toxicity as it is discharged to the receiving stream. The system is used as an early warning indicator of water quality conditions that may exceed acceptable discharge limits set by state and federal law. The system continually monitors the ventilation and body movement patterns of fish. Early warning stress is characterized by statistically significant changes in ventilatory and movement patterns and is used to identify developing acute toxicity of the effluent.

The University of Maryland (UMD) in collaboration with USACEHR evaluated the operation of the system for one year after the initial start-up mode was completed (second quarter of CY95) by USACEHR (Burton and Tieman, 1996). The UMD continued to provide operational assistance to USACEHR through the third quarter of CY99. The biomonitoring system was design to operate on-line 24 h a day, seven days a week. Upon completion of the start-up mode, the system operated on-line 97% of the time in CY95 and 99% of the time each year from CY96 through CY98. The percentage of time the system detected a stress event (primarily water quality changes) in CY95 through CY98 has been 8.2, 6.0, 2.3, and 1.1%, respectively. The on-line operational and percentage stress events in CY99 have not been determined at the writing of this report.

A significant aspect of operation of the biomonitoring system has been its positive effect on the management of the GWTF operation. After an initial learning period when the biomonitoring system was adapted to the specific operational conditions at the GWTF, the engineers at the facility became more aware of effluent conditions. As a result, the biomonitoring stress alarms have not unduly disrupted effluent treatment operations. Historically, the system facilitated signing of the Record of Decision for the site because it showed that the groundwater could be safely treated before discharge. A cost-benefits analysis estimated that the system initially saved $300,000 to $400,000 by eliminating treatment studies requested by EPA and State of Maryland regulators.

5. TOXICITY EVALUATION OF SELECTED SITES DURING HIGH SURFICIAL AQUIFER FLOW AT J-FIELD

The surficial aquifer in the vicinity of the toxic burning pits (TBP) at J-Field, Aberdeen Proving Ground, Maryland, is contaminated with a number of chlorinated aliphatic hydrocarbons. Groundwater flow measurements at J-Field have shown that the flow is generally from topographically high areas to topographically low areas. In the TBP area, the shallow water table has a local high in the area between the main burning pits and the Prototype Building. The steepest hydraulic gradient in the TBP area is to the southeast toward the marsh.

The major influences on the surficial aquifer flow system are recharge by infiltration of precipitation, evapotranspiration, and tidal fluctuations. Effective recharge appears to be greatest in the winter and least in the summer. The water level in the surficial aquifer in the TBP area is over 1.8 m (6 ft) above mean sea level (MSL) during a wet springtime and approximately 0.3 m (1 ft) below MSL during August and September when high evapotranspiration rates prevail. During the summer, flow reversals may take place with flow from the tidal estuaries providing recharge to the aquifer. Thus, one may infer that the southeast flow of contaminants into the marsh during these periods may be considerably reduced.

A series of aquatic bioassays were conducted at J-Field during the spring of 1994 and 1995 on surface water and surficial sediments obtained from areas likely to receive surface water runoff from the TBP area. Toxicity was detected by several bioassay systems in the TBP area of the marsh east of the TBP Pushout Area where the steepest hydraulic gradient of the surficial aquifer occurs. The current study by Burton and Turley (1997) was designed to define potential toxicity to the marsh ecosystem that may occur as a result of changes in the seasonal flow pattern of the contaminated surficial aquifer discharge into the marsh under high and low aquifer flow conditions (i.e., spring and late summer). The high aquifer flow phase of the study was completed as scheduled. However, the low flow phase could not be conducted because the marsh was dry during the low flow period (lowest rainfall in 30 years).

Aqueous phase bioassays were conducted on marsh surface water samples taken from three sites (SW-10, SW-11, and SW-12) east of the TBP Pushout Area and groundwater from a highly contaminated well upgradient from the TBP area (well JF8-3). A sediment phase bioassay was conducted on a composite sediment sample taken from the middle (SW-11) of the three surface-water sites. A sediment bioassay was also run on sediment taken from South Beach located due south of the TBP area approximately 15 m (50 ft) offshore (<1 m deep MSL). Comprehensive chemical and explosive analyses were also performed on all media analyzed for toxicity. The 1997 spring results of this study were compared to the toxicity and chemical data from previous spring studies at the

same sites.

Definitive acute aqueous phase bioassays were conducted with a cladoceran (*Ceriodaphnia dubia*), fathead minnow (*Pimephales promelas*), and African clawed frog (*Xenopus laevis*). The results of the assays were quite consistent with the screening and definitive bioassays conducted in the spring of 1994 with a cladoceran (*Daphnia magna*), fathead minnow, and southern leopard frog (*Rana sphenocephala*). Both studies showed that the surface waters at the three sites were not acutely toxic to cladocera and frogs. Two of the three sites (SW-10 and SW-12) were not acutely toxic to fathead minnow. The third site (SW-11) was not found to be toxic in the 1994 study during a 48-h screening test; a definitive acute test conducted in 1997 showed that the surface water was toxic to fathead minnow after a 96-h exposure.

Chronic toxicity tests conducted during the spring of 1997 on surface water from the same three sites showed the following. Briefly, both the 1994 and 1997 studies showed that the surface waters at the three marsh sites (SW-10, SW-11, and SW-12) in the vicinity of the TBP Pushout Area are chronically toxic to the green alga *(Selenastrum capricornutum*). Two of the three sites (SW-10 and SW-11) did not cause any chronic toxicity in 1997 to *Ceriodaphnia*; SW-12 was toxic during a 7-d exposure. No chronic toxicity to *Ceriodaphnia* was found at the three sites in the 1995 study.

All three sites caused chronic toxicity to the fathead minnow in 1997. Chronic toxicity was also found in the 1994 study at SW-10; chronic toxicity to the fathead minnow was not found at SW-11 and SW-12 in 1994. As stated above, the marsh surface waters at SW-10, SW-11, and SW-12 were not acutely toxic to frogs. Although the waters from the three sites did not cause significant mortality to frogs, significant increases in frog embryo malformations were found in teratogenicity assays at all three sites in 1997.

Surficial sediment taken from SW-11 was not toxic to the amphipod *Hyalella azteca* during a 28-d chronic exposure in the current study. A 28-d sediment study conducted in 1995 with the same species of amphipod found that SW-11 sediment was toxic (reduction in growth occurred). As in the current 28-d study, a 10-d acute sediment test conducted in May 1994 also showed that SW-11 sediment was not toxic *H. azteca*. Chronic sediment tests run in 1995 showed that sediments from SW-10 and SW-12 were not toxic to *H. azteca* during 28-d exposures. Sediment from SW-12 was found to be toxic to the amphipod in 1994 during a 10-d acute test.

Sediment from South Beach did not cause any chronic toxicity to the amphipod *(H. azteca*). EPA conducted a 10-d toxicity test with the saltwater amphipod *Ampelisca abdita* on sediment taken in August 1992 from the same general area as the current test. Although 35% of the treatment organisms died during the test, EPA concluded that the mortality was probably related to habitat preference (i.e., physical sediment variables) rather than potential toxicants bound to organic carbon. Screening level bioassays (May

to June 1993) on pore water taken from sediments in the same general area of South Beach have also shown that the sediments are not toxic.

As expected, the contaminated groundwater from the well (JF8-3) upgradient from the TBP marsh area was found to be acutely and chronically toxic to all organisms tested in 1997. Likewise, the 1994 study also showed that groundwater from the same well caused acute toxicity to the alga, cladoceran, fathead minnow, and frog.

Comprehensive chemical analyses and munitions analyses were performed on surface water taken from SW-10, SW-11, and W-12; groundwater from well JF8-3; and sediments taken from SW-11 and South Beach. The comprehensive chemical analyses included general chemistry, metals, volatile organics, base neutrals, acid compounds, pesticides/PCBs, and herbicides. Nitroaromatics and nitramines were determined in the explosive analyses. The results of the chemical analyses conducted in the current study were very similar to the results reported in prior studies for the three marsh sites, well JF8-3, and South Beach.

In summary, the current study supported the toxicological findings of previous studies. With regard to the marsh adjacent to the TBP Pushout Area, earlier screening studies did not identify toxicity to frogs. The current study showed that statistically significant mortality did not occur to frogs; however, malformations occurred at all Pushout Area marsh sites. Likewise, the current study showed that short-term chronic toxicity occurred to fish at two of three sites that in the past were not found to be chronically toxic to fish. The most heavily contaminated well upgradient from the Pushout Area contained toxic concentrations of chlorinated VOCs; however, chemical data from the current study for the marsh sites indicated that heavy metals were the primary contaminants of concern in the Pushout Area boundary of the marsh. Argonne National Laboratory's Focus Feasibility Study of the TBP site indicated that the source of heavy metals in the Pushout Area of the marsh was primarily from surface runoff from the adjacent contaminated soils. The current study supported Argonne National Laboratory's draft Remedial Investigation conclusion that ecological receptors are at risk in the marsh adjacent to the TBP Pushout Area. To date, the Army has performed soil removal actions in the TBP Pushout Area and is continuing an investigation of the use of phyto-remediation techniques to reduce the high VOC concentrations in the groundwater.

6. TOXICITY OF DIISOPROPYL METHYLPHOSPHONATE (DIMP) TO AQUATIC ORGANISMS AT THE BUILDING E3640 PROCESS LABORATORY SITE

Diisopropyl methylphosphonate (DIMP) is present in the subsurface soils and surficial groundwater in the vicinity of the Building E3640 Process Laboratory at the U.S. Army Aberdeen Proving Ground-Edgewood Area, Aberdeen, Maryland. DIMP has been found in two surficial wells downgradient of a surficial groundwater divide where the groundwater flow is to the north toward Kings Creek. The current study was initiated to determine the acute and chronic toxicity of DIMP and the possible interactions of DIMP with other contaminants that may be present in the groundwater as it enters the aquatic environment. The toxicity of DIMP and its interaction with other contaminants in the most highly contaminated well at the site (well # CCJ153A) was investigated as a worst case condition in the surficial aquifer north of the Building E3640 Process Laboratory. The toxicity of the parent compound was also determined to confirm the acute toxicity data in the literature and to provide chronic toxicity data which were not available.

The acute and chronic toxicity of DIMP and the possible interactions of DIMP with other contaminants that may be present in the groundwater and the parent compound were both evaluated using an array of four biomonitoring systems which included a number of endpoints. The biomonitoring assays covered several levels of biological complexity to maximize the predictability of DIMP toxicity to aquatic organisms. The following toxicity tests were conducted: 96-h green algal (*Selenastrum capricornutum*) growth test; 7-d cladoceran (*Ceriodaphnia dubia*) survival and reproduction test; and 7-d larval fathead minnow (*Pimephales promelas*) survival and growth test. In addition, survival and developmental toxicity were determined by the 96-h frog embryo teratogenesis assay-*Xenopus* (FETAX) using the African clawed frog, *Xenopus laevis*.

The concentrations of DIMP in the surficial groundwater samples taken from well CCJ153A on March 16, 18, and 20, 1998 at the Building E3640 Process Laboratory site, were 6.05, 5.09, and 4.72 mg/L, respectively. Low concentrations of several priority pollutant heavy metals (aluminum, barium, chromium, copper, and manganese) and one volatile organic (vinyl chloride) were also present in one or more of the three groundwater samples. No base neutrals, acid extractables, organophosphorus pesticides, or chlorinated pesticides and herbicides were found in the groundwater above the detection limits for drinking water. No nitroaromatic or nitramine explosives above a detection limit of 50 μg/L were present.

The groundwater was not acutely toxic to the green alga, cladoceran, or larval fish. The groundwater was not acutely toxic to frog embryos after 96 h of exposure. A statistically significant (alpha = 0.05) effect was found for frog embryo malformations; however, the effect was judged to be statistical error because the concentrations in the

groundwater were more than two orders of magnitude lower than the lowest-observed-adverse-effect level (malformation effect) established for the parent compound. The acute values for the alga, invertebrate, and larval fish species in this study fell within the range of acute values established in a prior study for several other freshwater species. As was the case for acute toxicity, the groundwater did not cause any short-term chronic toxicity to the green alga, cladoceran, or larval fish. The frog embryo test is an acute test; thus, chronic data were not obtained for the frog.

The concentrations of DIMP in the groundwater were found to be an order of magnitude or lower than the no-observed-adverse-effect levels (NOAEL) for short-term chronic exposure to the parent compound. The NOAELs for the alga (reduction in growth), invertebrate (reduction in neonate production), frog embryo (mortality), and larval fish (reduction in growth) were 711, 142, 398, and 142 mg/L, respectively. Based of the NOAELs, it was concluded that the apparent plume of DIMP migrating in the surficial aquifer from the Building E3640 Process Laboratory area towards Kings Creek should have minimal impact on the aquatic environment. The toxicity data obtained in the current study were used as part of a data base for a screening-level ecological risk assessment which is presented in the next section of this report. A draft Supplemental Report of DIMP toxicity has been completed by Burton and Turley (1999) and will be submitted for publication in the Department of Defense Technical Reports Database as a Supplemental Report to the contract.

7. SCREENING-LEVEL ECOLOGICAL RISK ASSESSMENT OF DIISOPROPYL METHYLPHOSPHONATE (DIMP) AT THE BUILDING E3640 PROCESS LABORATORY SITE

Diisopropyl methylphosphonate (DIMP) was found in the vicinity of the Building E3640 Process Laboratory (Building E3640) site during the 1994-1995 Remedial Investigation/ Feasibility Study (RI/FS) for the Canal Creek Study Area of the Aberdeen Proving Ground - Edgewood Area, Aberdeen Proving Ground, Maryland. DIMP occurred in 13 of 15 subsurface soil samples (vertical extent of contamination was evaluated from below the top soil horizon to the water table) in the northeast corner of the site. The concentrations ranged from a minimum of 0.07 up to a maximum of 4.8 mg/kg (dry wt.). DIMP was also found in two surficial wells downgradient of a surficial groundwater divide where the groundwater flow is to the north toward Kings Creek. The concentrations of DIMP were 0.08 and 2.17 mg/L in the surficial wells CCJ152A and CCJ153A, respectively. Subsequent analyses of three groundwater samples taken from well CCJ153A in 1998, showed that DIMP concentrations ranged from 4.72 to 6.05 mg/L.

The RI/FS concluded that 1) the DIMP in the subsurface soil is moving into the underlying surficial aquifer and 2) an apparent plume of DIMP is migrating in the surficial aquifer from the Building E3640 area towards Kings Creek. As a result, the U.S. Environmental Protection Agency (EPA) Region III recommended that additional information concerning the ecological hazard of DIMP be obtained for the site. A screening-level risk assessment was conducted to predict the likelihood of adverse ecological effects of DIMP in the subsurface soil at the site as well as the potential for adverse ecological effects as the compound moves in the surficial groundwater toward the Kings Creek area.

Two exposure pathways exist by which DIMP may reach the ecological receptors in the Building E3640 and Kings Creek areas. The first is the contaminated subsurface soil located in the spill area in the northeast corner of the Building E3640 site. The second potential exposure pathway is the apparent plume of DIMP which is migrating in the surficial aquifer from the Building E3640 area north northeast towards Kings Creek. All activities at Building E3640 which contaminated the soils and surficial aquifer were stopped in 1978. No DIMP is currently stored on site. Thus, no further releases of DIMP at the site will occur. Additional releases of DIMP to the groundwater could occur via the subsurface soils (infiltrating precipitation) in the spill area.

The following endpoints were used to evaluate the ecological risk of DIMP to the populations/communities in the Building E3640 and Kings Creek areas: 1) adverse effects to microorganisms, invertebrates, and plant communities from direct contact with DIMP in the soil; 2) adverse effects to aquatic life from exposure to DIMP in the sediment and water column; and 3) adverse effects to wildlife from the ingestion of material containing DIMP.

All no-observed-adverse-effect level (NOAEL) data used in the screening-level ecological risk assessment were based on conservative or worst case assumptions. A major assumption was made that the surficial aquifer would ultimately transport DIMP to the palustrine wetlands and tidal wetlands of the Kings Creek area as well as Kings Creek. No evidence exists which shows that DIMP has in fact moved from the Building E3640 site. Likewise, the assumption was made that DIMP concentrations in the surface and subsurface soils in the palustrine wetland areas and tidal wetland sediments were the same as the highest concentration in the surficial aquifer since the surficial aquifer would be the source of the contaminant. To be conservative, 6.02 mg/kg was used as a worse case for all soil calculations even though the highest DIMP concentration in the subsurface soils at the Building E3640 site was 4.8 mg/kg. The bioconcentration data for plants (agricultural plants) reported in the literature and used in the risk assessment are questionable (data not treated statistically). However, the highest BCF (10.7) for agricultural plants was used for all plants in the risk assessment. The worst case assumption was made that DIMP was bioconcentrated to 64 mg/kg dry weight (10.7 [BCF] x 6.02 mg/kg = 64) in the roots, stems, and leaves of all plants in the Building E3640 and King Creek areas.

The assumption was made that the highest concentration of DIMP found in the surficial aquifer (6.02 mg/L) at the Building E3640 site was in equilibrium with the bulk sediment, sediment interstitial water and water column in Kings Creek. Thus, any organism present in Kings Creek was assumed to be exposed to a maximum concentration of 6.02 mg/L. The worse case assumption was made that the oral exposures for all wildlife in both the Building E3640 and Kings Creek areas would be 64 mg/kg/d. All wildlife found in the study area consume some plant material in their diet. Species which consume multiple food types, such as, a mixed diet of plants and animals would not consume DIMP at a rate of 64 mg/kg/d because animals do not accumulate DIMP above background levels.

A number of exposure-modifying factors (e.g., home range, season, behavior, etc.) can modify wildlife contaminant exposure. The assumption was made that all organisms were always in contact with the contaminant at the maximum concentrations given above. The assumption was also made that DIMP was 100% bioavailable to all receptors at all times. DIMP does not accumulate above background in animals because of its low log octanol water partition coefficient (1.03); thus, DIMP bioconcentration/ bioaccumulation in animals was not considered in the risk assessment. When chronic NOAEL data were not available for use in the risk assessment calculations, established uncertainty factors were used to estimate NOAELs from subchronic values.

No data are available regarding the modes of DIMP toxicity to organisms in most plant and animal phyla. Toxicokinetic studies have shown that DIMP is rapidly absorbed (15 min to 3 h depending on the species) following oral administration in mammals. DIMP is initially distributed throughout the body via the circulatory system, followed by high

concentrations primarily in the liver, kidneys, and urinary bladder in 4 to 24 h after oral administration. DIMP is metabolized primarily to isopropyl methylphosphonic acid (IMPA); some hydrolysis of IMPA to methylphosphonic acid (MPA) may occur in the liver or in other tissues. Peak urinary excretion of a single dose occurs between 6 and 72 h depending on the species. No storage of DIMP, IMPA, or MPA occurs in the body of mammals, although portions of [^{3}H]-label may become incorporated in biomolecules leading to some retention of label in the form of unextractable labeled compound. DIMP is not genotoxic or carcinogenic to birds or mammals (including humans) after oral exposure. DIMP is not a developmental hazard to larval frogs.

Screening-level ecological risk calculations show that DIMP poses a negligible risk to plants and animals in the Building E3640 Process Laboratory site and Kings Creek areas. The conclusion was based on the fact that the hazard quotients (HQ) of all potential receptors in the study area were <1. The HQs for soil microorganisms, soil and litter invertebrates, and terrestrial plants were estimated to be 0.06, 0.05, and 0.60, respectively. The HQs for aquatic microorganisms (bacteria), aquatic algae, aquatic invertebrates, fish, and amphibians were 0.06, 0.01, 0.10, 0.04, and 0.02, respectively. The HQs for birds and mammals were 0.63 and 0.76, respectively. No DIMP data were available for reptiles; thus, an HQ was not calculated for reptiles. Based on the data for other vertebrates, the weight-of-evidence suggests that DIMP will not be a risk to reptiles.

At the writing of this report, the U.S. Environmental Protection Agency Region 3 and the State of Maryland Department of the Environment have agreed with the Army that DIMP poses no ecological hazard to the environment. A draft Supplemental Report has been prepared and will be submitted for publication in the Department of Defense Technical Reports Database as a Supplemental Report to the contract (Burton, 1999).

8. BIOACCUMULATION OF TOTAL MERCURY AND METHYLMERCURY IN EARTHWORMS AND THE ECOLOGICAL RISK TO BIRDS AND MAMMALS AT GRACES QUARTERS' NORTHEAST TEST HUT

Mercury-contaminated soil was found in the vicinity of the Northeast Test Hut during the 1993 -1995 Remedial Investigation of Graces Quarters, Aberdeen Proving Ground, Maryland. Additional sampling in 1997 established the spatial extent of the contamination. The contamination of the site appears to have occurred during chemical warfare materiel decontamination studies conducted during the 1950s. The concentrations of total mercury in the soil (0-15 cm horizon; 0-6") ranged from ~0.1 mg/kg dry soil (background) up to ~15 mg/kg dry soil. An ecological risk assessment of the mercury contamination, which assumed that all mercury at the site was methylmercury (most toxic form of mercury to animals), indicated that the potential exists for adverse effects to wildlife. Because of the contamination at the site, the U.S. Environmental Protection Agency (EPA) Region III established a clean up level for total mercury of 0.1 mg/kg dry soil (background level) for Graces Quarters. However, EPA Region III gave the Army the option of conducting bioaccumulation studies with earthworms exposed to contaminated soil at Graces Quarters in order to determine the bioaccumulation factors for methylmercury which could be used to more accurately assess the risk to wildlife which may feed on earthworms at the site. In addition to the bioaccumulation evaluation, the ecological risk to the American robin (*Turdus migratorius*) and short-tailed shrew (*Blarina brevicauda*) which feed on earthworms exposed to mercury, was evaluated in the study.

Three contaminated soil samples were taken in the vicinity of the Northeast Test Hut for the bioaccumulation study. They included soil with the highest concentration of total mercury at the site, an intermediate contaminated soil, and a low total mercury soil. A reference soil sample was taken approximately 120 m (~400') east of the Graces Quarters entrance gate approximately 15 m (~50') inside the boundary fence. All soils, which were Mattapeake/Mattapex soil, were taken from the 0-15 cm (0-6") soil horizon. The concentrations of total mercury in the high, intermediate, low, and reference soils were 11,542, 2,824, 156, and 85 ng/g dry soil, respectively. The concentrations of monomethylmercury (MMHg) were 7.35, 2.56, 1.48, and 1.12 ng/g dry soil, respectively, in the high, intermediate, low, and reference soils. MMHg was studied because it is the dominant form of methylmercury in soil. The lumbricid earthworm *Eisenia fetida* was used for all bioaccumulation tests.

Bioaccumulation was determined by the steady state method. The experimental design consisted of a 28-d uptake phase in the three contaminated soils and reference soil followed by a 28-d depuration phase in reference soil only. During the uptake phase, four randomly selected replicates of 10 worms/replicate were analyzed for total mercury and MMHg in each contaminated soil and reference soil at days 0, 1, 2, 4, 7, 14, 21, and 28. Four randomly selected replicates of 10 worms/replicate were analyzed for total mercury

and MMHg from each contaminated soil and reference soil at days 35, 42, 49, and 56 during the depuration phase. At each sample period, the worms in each replicate were counted, their guts purged for 24 h, and weighed. At the end of the 28-d uptake phase, the worms in each remaining replicate were counted, their guts purged for 24 h, and weighed. Earthworms in all remaining replicates were placed in clean reference soil and sampled at the frequency described above during the 28-d depuration phase.

The bioaccumulation of total mercury and MMHg at all treatments was estimated by the BIOFAC model which treats each organism as a single compartment. The model was initially used to estimate the rate constants characterizing the uptake and depuration of both materials. BIOFAC consists of a nonlinear parameter estimation routine which generates optimal estimates of the rate constants from a set of sequential time-concentration data. The data were weighted by a normality preserving transformation to reflect any time- or concentration-related trends in variability. Uncertainty in the parameters as well as the validity of the model were also estimated by the model. The uptake (k_1) and depuration (k_2) rate constants were used to calculate the bioaccumulation factor (BAF) of each material at each treatment. In addition to the uptake (k_1) and depuration (k_2) rate constants and the BAFs, BIOFAC was also used to estimate time to reach 90% of steady state.

The uptake of total mercury and MMHg at all four treatments (reference soil also contained mercury) followed a one-compartment bioaccumulation plateau model; however, uptake did not reach equilibrium in 28 d. The degree of curvature of the negative curvilinear uptake curves (negative curvature indicates that the rate of accumulation slowed over time) was not significantly different among the four treatments for both total mercury and MMHg which indicates: (1) the uptake of the chemical is directly proportional to the exposure concentration in the soil; thus, whatever is governing the uptake of the particular chemical is similar across all treatments and (2) the uptake rate constant(s) (k_1) will be independent of the exposure concentration.

The depuration of total mercury and MMHg did not follow one-compartment bioaccumulation depuration kinetics. In the case of total mercury, the high treatment decreased from day 28 to day 42 and appeared to reach a steady state. The intermediate and low treatments decreased through day 42 but then reversed direction and increased with time. The total mercury reference treatment did not decrease during depuration; it continued to increase and reached a steady state in approximately 40 d. With regard to MMHg, the high, intermediate, and low treatments initially decreased through day 42 of depuration, but then changed direction and increased throughout the remainder of the depuration period. The reference MMHg treatment did not decrease but continued to increase throughout the depuration period. The lack of "classical" depuration is most likely due to the presence of total mercury and MMHg in the reference soil during depuration. In "normal" one-compartment bioaccumulation models, the shape of the curve during depuration in contaminant-free soil is essentially the same for all treatments when the

concentrations of chemical in the animals are different at the initiation of depuration. In the current study, all four treatment groups were placed in reference soil which contained both total mercury and MMHg during the depuration phase; thus, the earthworms were continually exposed to the contaminants during the depuration phase.

Growth within both the uptake and depuration phase was linear. No difference occurred between treatments within the uptake and elimination phases. Thus, concentrations of total mercury and MMHg as high as 11,542 and 7.35 ng/g dry soil, respectively, did affect the growth of the earthworms. A transient laboratory handling effect, however, occurred at the beginning of both the uptake and elimination phases.

Total mercury bioaccumulation factors (BAF) were estimated as follows. The total mercury concentrations in the high, intermediate, and low treatments at day 42 of depuration were used as the k_2 estimates for the model. All data through day 56 were used for the reference BAF estimate since the worms were held in a constant exposure for 56 d. The BAFs for total mercury in the high, intermediate, low, and reference treatments were 0.8, 0.6, 6.0, and 3.7, respectively. The estimated times to reach 90% of steady state in the high, intermediate, low, and reference treatments were 40, 41, 137, and 36 d.

The BAFs for MMHg were estimated using the day 42 depuration data in the high, intermediate, and low treatments as the k_2 estimates in the BIOFAC model. The reference treatment was estimated using the uptake data only. The BAFs for MMHg were 175, 188, 234, 262, respectively, in the high, intermediate, low, and reference treatments. The time to reach 90% of steady state for the high, intermediate, low, and reference treatments were estimated to be 172, 92, 97, and 212 d, respectively

The ecological risk to robins and shrews consuming contaminated earthworms was estimated for MMHg which is the most toxic form of mercury in soil. Briefly, the exposure assessment was performed by calculating the potential ingestion of MMHg via ingestion of food (earthworm) and contaminated surface soil at the Northeast Test Hut. The highest MMHg BCF (262) was used for all calculations. Two diets were used to calculate the potential ingestion of MMHg in the robin and shrew. The first scenario assumed that 100% of the robins's and shrew's total diet was comprised of earthworms. The proportion of diet consisting of earthworms was also evaluated in a less conservative, but more realistic scenario. In the second scenario, diets of 22% earthworm for robins and 32% for shrews were used based on data for robins and shrews living in the mid-Atlantic region. The total daily dietary exposure doses for the robin consuming diets of 22% and 100% earthworms at the high site were 0.009 and 0.040 mg/kg bw-d; 0.003 and 0.014 mg/kg bw-d at the intermediate site; 0.002 and 0.008 mg/kg bw-d at the low site; and 0.001 and 0.006 mg/kg bw-d at the reference site, respectively. The total daily dietary exposure doses for the shrew consuming diets of 32% and 100% earthworms at the high, intermediate, low, and reference sites were 0.038 and 0.120 mg/kg bw-d; 0.013 and 0.042 mg/kg bw-d; 0.008 and 0.024 mg/kg bw-d; and 0.006 and 0.018 mg/kg bw-d, respectively.

The estimated daily dietary doses for the robin and shrew were compared with the Graces Quarters ecological effects assessment toxicity reference values (TRVs) established from the literature by The IT Group (Edgewood, Maryland). The TRVs, which are assumed to be protective of the ecological receptors being evaluated, are 0.012 and 0.151 mg/kg bw-d, for the robin and shrew, respectively. The daily dietary MMHg doses for the robin and shrew at consumption rate of 22% and 32 % earthworm, respectively, are below the TRVs for both species at all Northeast Test Hut soil concentrations. At a 100% earthworm diet, the robin daily MMHg dietary exposure would be exceeded at the intermediate and high soil concentrations. The daily dietary dose for shrews would not be exceeded at any site if the animal's diet consisted of 100% earthworm.

In the risk characterization process, the potential exposure concentrations are compared with the TRVs to evaluate the potential for adverse effects to the ecological resources from exposure to the contaminant of potential concern. Estimated exposure concentrations are compared to TRVs by creating a ratio of the estimated exposure concentration to the TRV. The ratio is termed the environmental effects quotient (EEQ). If the EEQ is <1.0 then adverse effects are considered unlikely; conversely, if the EEQ is >1.0 there is a potential for adverse effects to occur. The EEQs for the robin consuming diets of 22% and 100% earthworms at the high, intermediate, low, and reference sites are 0.75 and 3.33; 0.25 and 1.17; 0.67 and 0.17; and 0.50 and 0.08, respectively. The EEQs for the shrew consuming diets of 32% and 100% earthworms at the high, intermediate, low, and reference sites are 0.25; and 0.79; 0.09 and 0.28; 0.05 and 0.16; and 0.04 and 0.12, respectively.

The EEQs for the robin at a 100% earthworm diet indicate that the potential for adverse effects exists if the robin consumes earthworms which bioaccumulate MMHg at the intermediate and high soil sites at the Northeast Test Hut. The EEQs at the low and reference sites indicate that no adverse effects will occur when the diet is 100% earthworms. When one considers the less conservative, but more realistic diet of 22% earthworms, the EEQs for all soil concentrations indicate that no adverse effect is likely to occur at the Northeast Test Hut. The EEQs for the shrew at both the conservative diet of 100% earthworms and less conservative diet of 32% earthworms are all <1 which indicate that the potential for adverse effects from MMHg in the soils is very low. The data from this study show that no remedial action is necessary at the Northeast Test Hut area based on robins consuming a diet of 22% earthworm and shrew consuming a diet of 32% (and 100%) earthworms contaminated with MMHg at the highest soil concentration at the site.

The results of this study are in a draft report currently being reviewed by the EPA Region 3 and the State of Maryland Department (Burton and Turley, in review). The report will be submitted as a Supplemental Report for publication in the Department of Defense Technical Reports Database when the final version is approved by the above regulatory agencies.

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